Mucus Associated Proteins and Their Functional Role in the Distal Intestine

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Ragnar Sandberg, Medicinaregatan 7A, Göteborg,
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Av

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Avhandlingen baseras på följande delarbeten:

I. Rodríguez-Piñeiro AM, Bergström JH, Ermund A, Gustafsson JK, Schütte A, Johansson MEV, Hansson GC. (2013) **Studies of mucus in mouse stomach, small intestine, and colon. II. Gastrointestinal mucus proteome reveals Muc2 and Muc5ac accompanied by a set of core proteins**. Am J Physiol Gastrointest Liver Physiol. 1;305(5):G348-56.

II. Bergström JH, Berg KA, Rodríguez-Piñeiro AM, Stecher B, Johansson MEV, Hansson GC. (2014) **AGR2, an Endoplasmic Reticulum Protein, Is Secreted into the Gastrointestinal Mucus**. PLoS One. 11;9(8):e104186.

III. Bergström JH, van der Post S, Schütte A, Ermund A, Johansson MEV, Hansson GC, Bäckström M. **The von Willebrand D domains in the mucus associated protein FCGBP are autocatalytically cleaved during early biosynthesis**. *Manuscript*

IV. Bergström JH, Gustafsson IJ, Johansson MEV, Hansson GC. **ZG16 is secreted by goblet cells and bind enterocytes**. *Manuscript*

V. Bergström JH, Birchenough GM, Katona G, Schütte A, Ermund A, Johansson MEV, Hansson GC. **Gram positive bacteria are held at a distance in the colon mucus by the lectin-like protein ZG16**. *Manuscript*

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ABSTRACT

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The mammalian intestine, especially the large intestine, harbors complex societies of beneficial bacteria coexisting with the host. This is a mutualistic relationship, where the host provides nutrients and a favorable environment while the bacteria in return ferment indigestible polysaccharides to short chain fatty acids. The host needs to keep the bacteria at a safe distance from the intestinal cells to prevent disease. The first line of defense hindering these microorganisms from invading the underlying epithelium is a mucus layer built by the highly polymeric and heavily *O*-glycosylated MUC2 mucin, the main structural component. In the colon this mucus barrier is made up of two layers with similar composition. The outer layer is loose and easy removable while the inner is more dense and firmly adherent to the underlying epithelium. The inner layer is impermeable to bacteria and therefore separates the bacteria that reside in the lumen from the epithelial cells. In order to obtain a better understanding of the function and structure of this dynamic barrier we analyzed the mucus using proteomics based approaches to identify novel mucus components

The focus of this thesis has been trying to understand the specific protective role of the MUC2 assosiated proteins in the mucus layer. Three proteins were chosen for further studies based on their abundance and production by the mucus secreting goblet cell.

AGR2 belongs to the protein disulfide isomerase family, and has been proven important for proper MUC2 production. Using molecular biology tools and cell culture experiment it was shown that AGR2 does not covalently bind the MUC2 terminal recombinant proteins and that secretion of the molecule is dependent on an internal cysteine residue.

The mucin-like protein FCGBP is a highly repetitive molecule that contains 13 von Willebrand D domains. Eleven of these contain an autocatalytic cleavage site that forms a new reactive C-terminus after cleavage, which occurs early during biosynthesis.

ZG16 is a lectin-like molecule that has now been shown to bind peptidoglycan, the major bacterial cell wall component, via its carbohydrate recognition domain. It was shown that ZG16 is not bactericidal, but that it binds and aggregates Gram-positive bacteria and translocate them further out in the mucus. ZG16 is also able to bind to enterocytes via a protein receptor implying a novel sensory function.

In summary, the results from this thesis demonstrate that these MUC2 associated proteins are important to form a functional protective mucus layer that prevents bacteria to reach the epithelium and by this cause disease.

Keywords: intestine, mucus, bacteria, MUC2, AGR2, FCGBP, ZG16
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